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14. ABSTRACT
Compared with other serum polypeptides, autoantibodies have many appealing features as biomarkers including sensitivity, stability, and easy detection. Anti-lipid autoantibodies are routinely used in the diagnosis of autoimmune disease, but their potential for cancer diagnosis has not been explored. Dysregulation of cellular signaling in cancer cells would be expected to lead to irregular metabolism of many lipids, which could be sensed by immune system and cause the production of novel autoantibodies. Indeed, recent reports describe anti-lipid antibody production in cancer patients. Our hypothesis is that a broad and irregular change in lipid profiles in breast cancer cells results in the production of anti-lipid antibodies that could be used as biomarkers for early diagnosis. We propose to generate arrayed lipids on the membranes (lipid microarrays), and use them to examine global anti-lipid profiles at different stages of carcinogenesis in a transgenic breast cancer model. We have successfully set up the technology for lipid microarrays in our laboratory. Using fluorescently labeled secondary antibodies and an internal control labeled with a different fluorophor, we have greatly improved the sensitivity and reproducibility compared to the first generation of lipid microarrays. We are currently collecting more serum from newly bred mice at different ages.

15. SUBJECT TERMS
Breast cancer, serum biomarker, lipid microarrays, transgenic mouse model

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Introduction:

Breast cancer is the most common form of cancer among women. Compared with other serum polypeptides, autoantibodies have many appealing features as biomarkers including sensitivity, stability, and easy detection. Anti-lipid autoantibodies are routinely used in the diagnosis of autoimmune disease, but their potential for cancer diagnosis has not been explored. Metabolism of lipids immediately follows cellular stimulation, resulting in various lipid metabolites. Dysregulation of cellular signaling in cancer cells would be expected to lead to irregular metabolism of many lipids, which could be sensed by immune system and cause the production of novel autoantibodies. Indeed, recent reports describe anti-lipid antibody production in cancer patients. Our hypothesis is that a broad and irregular change in lipid profiles in breast cancer cells results in the production of anti-lipid antibodies that could be used as biomarkers for early diagnosis. We propose to generate arrayed lipids on the membranes (lipid microarrays), and use them to examine global anti-lipid profiles at different stages of carcinogenesis in a transgenic breast cancer model.

This is a high-risk proposal. However, the relevance to breast cancer is high and the potential reward is great. If the proposed experiments are successful, anti-lipid autoantibodies will be used as a new type of biomarker that could be used in conjunction with other biomarkers for early breast cancer diagnosis. Knowledge of the lipid changes would also help us understand breast cancer pathogenesis, and might thus lead to the design of new therapeutic strategies.

Body:

We have successfully set up the technology for lipid microarrays in our laboratory. Using fluorescently labeled secondary antibodies and an internal control labeled with a different fluorophor, we have greatly improved the sensitivity and reproducibility compared to the first generation of lipid microarrays. We are currently collecting more serum from newly bred mice at different ages. The major progresses are summarized below.

Improvement of currently existing lipid microarray methodology

In the original lipid array protocol, chemiluminescent detection was used to detect antibody reactivities to lipids and glycolipids spotted on PVDF membranes. Chemiluminescent detection relies on an enzymatic reaction that produces light, which is detected by a CCD camera or imaged on film. Some samples produce bright light for a short time, and others produce comparatively dim light, but for a long period of time. Therefore, images must be collected at an optimized time. This time-dependence of signal compromises quantification and accuracy. We decided to improve this technique by using a dual-labeled fluorescent detection, which can be detected by a LI-COR Odyssey instrument. The first two issues we set up to test were the membrane support and blotting solution. The PVDF membrane was used to spot lipids in the original lipid arrays. However, autofluorescence was consistently high on PVDF membrane when the fluorescently labeled secondary antibodies were used. We then decided to test if other membranes could lower the autofluorescent background. Nitrocellulose membrane had low background, but reacted to some solvents used to dissolve lipids (data not shown). We then chose to use a new type of PVDF from Amersham, Hybond-LFP, which the manufacturer claimed to have low fluorescent background on Western blotting. We compared the performance of regular PVDF and HyBond-LFP in three different blocking buffers. Different amounts of asialo-GM1 (200 pmol, 40 pmol, 8 pmol, 3.2 pmol and 0.64 pmol) were spotted on either Hybond-LFP or regular PVDF (BioRad). The membranes were blocked in BSA, casein, or Odyssey (from LI-COR) blocking buffers, detected by a polyclonal rabbit asialo-GM1 antibody and followed by IRDye 800 goat anti-rabbit (green channel) secondary antibody. Although no secondary antibodies were used for channel 700, the background in this channel is extremely high on PVDF membranes in all three blocking conditions (Fig 1. Right panel). However, the background in channel 700

is minimal on Hybond-LFP (Fig 1. left panel). The background in channel 800 is low and is very similar in all blocking solutions on both regular PVDF and Hybond-LFP. Among all blocking solutions, Odyssey blocking buffer gave the best sensitivity (Fig 1. bottom, left panel).

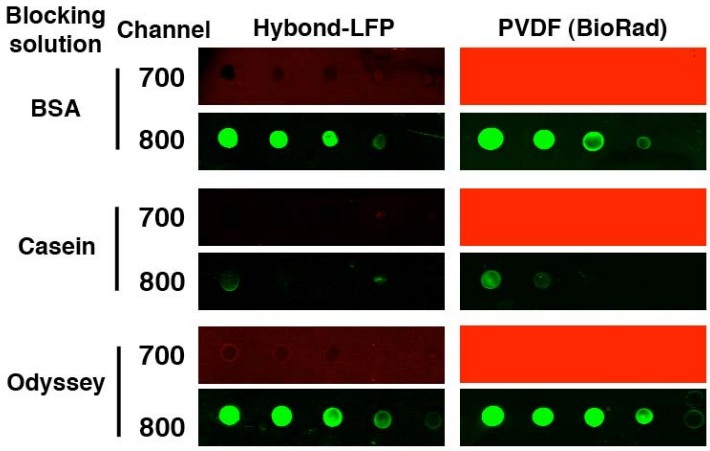

Fig 1. Improvement of lipid array methodology. Asialo-GM1 was serially diluted (200 pmol, 40 pmol, 8 pmol, 3.2 pmol and 0.64 pmol) and spotted on either Hybond-LFP or regular PVDF (BioRad). The membranes were blocked in BSA, casein, or Odyssey (from LI-COR) blocking buffers, then detected by a polyclonal rabbit asialo-GM1 antibody and followed by IRDye 800 goat anti-rabbit secondary antibody. The membranes were then scanned in both channel 700 (red) and channel 800 (green) using a LI-COR Odyssey instrument.

Array validation

We have validated our lipid arrays using polyclonal and monoclonal antibodies with defined specificities. The polyclonal antibodies specific for asialo-GM1 bound specifically to GM1, but not to the closely related gangliosides GM1 or GM2 (Fig. 2). Monoclonal antibodies raised against GD3 specifically bound GD3, but not to asialo-GM1, GM1 and GM2 (Fig. 2). The secondary antibodies did not show reactivity against lipids (data not shown).

Asialo GM1	GM1
GM2	GD3

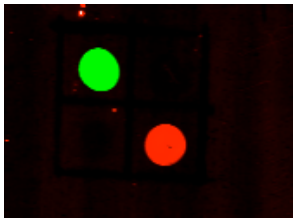

Fig 2. Lipids on the PVDF membrane can be detected by specific antibodies. 200 pmol of Asiao-GM1, GM1, GM2 or GD3 were each spotted on PVDF membrane. The membrane were then blocked in Odyssey blocking buffers, incubated with rabbit asialo-GM1 polyclonal and mouse monoclonal GD3 antibodies, and detected by Alexa 680 goat anti-mouse (red channel) and IRDye 800 goat anti-rabbit (green channel) secondary antibodies. Asiao-GM1 and GD3 antibodies have reactivity only to the corresponding lipids. No non-specific signal to irrelevant lipids was detected.

The specificity of lipid array and dual-color fluorescent labeling should allow us to label mouse serum samples using one color, and a well-characterized polyclonal rabbit antibody against an lipid (such as asialo-GM1) using another color simultaneously. The reactivity to asialo-GM1 can then be used as an internal control, allowing us to compare different membranes, and thus providing a better method to quantitate fluorescent intensity on different membranes.

Mice breeding and serum collection

We have successfully expanded our mice and started to collect serum from both wild-type control and transgenic breast cancer mice control. We expect that we will get some novel findings when this project is finished. However, delays in getting animal protocol approval and for the shipping of the parental transgenic breast cancer mouse model from Jackson laboratory have slowed our progress and lengthened the time required to complete the proposed experiments. As well, the breeding and expansion of the mice has taken a bit longer than we anticipated. At this point, it is becoming increasingly clear that we will need longer than the originally projected year to complete the funded work within the original approved project scope and objectives. W have accordingly requested for a no-cost extension of the award, which should suffice to address the delays we have encountered.

Key Research Accomplishments

- Set up a new lipid arrays methodology based on fluorescent detection. This change improved accuracy and dynamic range of lipid arrays.
- Validated the feasibility of lipid arrays
- Bred and expanded transgenic mice.
- Collected some serum samples from mice.

Reportable Outcomes

The funding has been used to support part of PI's and a technician (Yue Zeng)'s salaries. It has also provided research training for the technician, who is now enrolled in the medical school of Indiana University.

Conclusion

The new fluorescent detection would allow us to better compare the serum reactivities to lipids. We will expect that this project will be finished in the next year.

References

N/A

Appendices

None

Supporting data

None